frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 09 June 2023
DOI 10.3389/fsufs.2023.1190340

OPEN ACCESS

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RECEIVED 20 March 2023
ACCEPTED 18 May 2023
PUBLISHED 09 June 2023

CITATION
Moldagaliyeva D, Uzakov Y, Sarsembayeva N, Ibazhanova A, Jussipbekova B, Nurakhova A, Artykbayeva U and Baimuratova M (2023) Functional semi-finished fish product evaluation: organoleptic and evidence *in vivo*. *Front. Sustain. Food Syst.* 7:1190340. doi: 10.3389/fsufs.2023.1190340

Functional semi-finished fish product evaluation: organoleptic and evidence *in vivo*

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Introduction: The development of functional products is a new promising trend in the modern food industry. The research aims to confirm the quality indicators, efficacy and safety for living organisms of the developed functional semi-finished fish product—raw smoked sausage. The research was aimed at studying the characteristics of the semi-finished product obtained, including its organoleptic properties. However, it was also important to test the properties of the probiotic component added to the developed product and its effects *in vivo* on mice to verify the efficacy and safety of the *E. coli* 64 G strain.

Methods: Organoleptic and histological assessments of the product were performed. A series of experiments were also conducted to assess the product's safety and functional properties. For this purpose, Enteracol was fed to white outbred laboratory mice with different concentrations of the active ingredient (*Escherichia coli* 64G strain). Organoleptic properties of the proposed product, such as smell, consumer view, and balanced taste, demonstrated high consumer qualities of the crude smoked sausage with a probiotic component.

Results: The results of the controlled prospective study confirmed that the product is safe and non-toxic to living organisms: mice state alive after consuming a created meal. At the same time, assessing the product's antagonistic activity revealed its high protective effect (85%–100% survival rate of animals in the experimental groups compared to the control with 100% mortality).

Discussion: The proposed product has a stimulating effect on an organism and demonstrates the antagonistic activity against pathogenic and opportunistic bacteria established in the Enterobacteriaceae family members. The next step will be a long-term study of the product's stimulating effect to prove its positive impact on the body.

KEYWORDS

***Escherichia coli* 64G, Enterocol, semi-finished product, fish, survival**

1. Introduction

Food culture is critical in ensuring human health and is an essential and integral part of a healthy lifestyle. With this aim, functional feeding is optimal, providing nutrients, minerals, vitamins, and microorganisms that promote digestion and absorption of vital components (Alexandri et al., 2022). Consuming industrially processed foods and living a sedentary lifestyle result in excess calories. So, on the one hand, it is necessary to reduce the size of consumed portions and, on the other hand, to increase their nutritional value. This problem can be solved by enriching food products with nutrients necessary for the body's normal functioning. Functional nutrition is considered an alternative to drug therapy in the modern world, as functional products are developed to reduce cure and prevent disease (Gok and Ulu, 2019; Alexandri et al., 2022).

From a gerontological point of view, the risk of developing a functional immunodeficiency in older adults should be noted. In people over 60, the reduced activities of monomeric and dimeric immunoglobulin A (IgA) as well as T- and B-lymphocytes are fixed. Immunoreactivity disorder causes changes in the composition of mucosal biofilms and skin which raises chronic inflammation, infectious diseases, and autoimmune and oncological disorders (Abbas et al., 2019; Zaki et al., 2020). Probiotics have a positive effect on relieving mild inflammation and increasing immune reactivity. Probiotics and omega-3 fatty acids, as a part of therapy, contribute to the normalization of gut microbiota with a predominance of autochtone bacteria that positively affects the immunology reactivity (Hutchinson et al., 2020). Talan et al. (2001) give data that the consumption of probiotic-containing cheese contributes to improving natural cellular immunity in older volunteers due to an increase in the NK count and blood phagocytic activity. Data by Campmans-Kuijpers and Dijkstra (2021), as well as Zhou C, et al. (2020), gives us a possibility to say about the positive therapeutic effect of irritable bowel syndrome (IBS) treatment normalizes motor disorders and reduces visceral hypersensitivity. Unfortunately, the consumption of probiotics and functional foods in the Republic of Kazakhstan is low. Kazakhstanis consume less than 3 kg of functional products per person a year, whereas 20% of the Kazakh population are people over 60 years and this percentage is projected to increase to 25% by 2030 (Katsaga et al., 2012; Ashirova et al., 2021). Kazakhstan should therefore look to the experience of European countries, where functional products make up about 1/4 of the population's diet (Lyra et al., 2016). We should take the following steps in this regard. First of all, the functional product should be food, not pharmaceutical drugs. A probiotic product should have a natural structure and contain sufficient probiotic components (at least 1/5 of the body's daily requirement) (Lorimer, 2020). Its action must be safe and effective to prevent or cure some diseases, such as cardiovascular, gastrointestinal, and endocrine diseases (Battino et al., 2019; Angeles et al., 2021). Supplying the population of a given area with functional products using the scarcest nutrients in the region will also be great (Dyshluk et al., 2018).

Older people (around 70 years old) have some changes in their gut microflora compared to healthy adults. Arboleya et al. (2016) have found some differences in the content of *Collinsella*, *Clostridium leptum*, and *Eubacterium limosum*. And the number of bifidobacteria decreases with age compared to opportunistic facultative anaerobes. Various finished probiotic products, such as canned meats, fermented milk drinks, cottage cheese, and molded semi-finished fish products, can balance the intestinal microflora. However, the list of available specialised products, especially those produced in Kazakhstan, could be more extensive (Lorimer, 2020). Geriatrics data indicate that the fishes of the family Cichlid (*Cichlidae*) contain components with anti-ageing properties, and they can be recommended to elevated concentrations of glycine, chondroitin sulfate, hyaluronic acid, calcium phosphate salts, and phospholipids (Young, 2009). This recommendation was a basis for developing the technology for semi-finished fish products from tilapia (*Oreochromis niloticus*) for a geriatric-oriented diet.

Nile tilapia fillet is low in fat, rich in high-quality proteins, balanced amino acid composition and sufficient vitamins and minerals. This will expand the range of functional products, balanced in nutritional value and chemical composition (Young, 2009; Banning, 2011). With this in mind, we have developed a functional product in the form of a crude smoked sausage from *Oreochromis niloticus* cultivated in a closed reservoir with Enterocol use (an experimental biological product based on *Escherichia coli* 64G strain) (Zhakupova et al., 2017; Sarsembaeva et al., 2021b).

This study aims to assess a previously developed probiotic fish product to confirm its quality, effectiveness, and safety for living organisms.

2. Materials and methods

During the development of a functional product (crude smoked sausage), a controlled, prospective study was conducted in white mice to determine the efficacy and safety of fishmeal produced from fish flesh cultured with the Enterocol (*E. coli* 64G) probiotic. For control, Nile tilapia was grown in a closed reservoir under similar conditions and a similar diet but without using a biological preparation during the cultivation process (control group). The control group fish flesh was used to produce a similar semi-finished product (control product).

In manufacturing, we have enriched the created fish semi-finished product with probiotic supplements such as a mix of the *Streptococcus diacetilactis*, *Lactobacillus celobiosum* and *E. coli* (64G strain) (Sarsembaeva et al., 2021b).

The first stage was preparing the tilapia flesh (defrosting, deboning, and sinewing). In the second stage, the prepared fish flesh was salted for 12 h. Then it was cut into 2–3 mm pieces and mixed for 8–10 min with spices. At this stage, the probiotic component was added to the minced meat. The sausage sticks were then formed. The exposition time after this stage was 4 h, after which the thermic processing was conducted. First, the sausage sticks were roasted ($t = 60°C$; for 40 min) and then cooked ($t = 60°C$). The next step was cooling for 2 h ($t = 20°C$), after that—smocking for 12 h ($t = 43°C$). Then sausage sticks have been drying for 24 h at 10°C–12°C and air humidity of 76.5 points. The last phase was checking the sausage safety (microbial for the prime), packaging, and labelling the finished product.

Therefore, in our experimental research, we wanted to evaluate the proprieties of the created food product, which can be a part of functional feeding in the future, and to prove the safety of the probiotic supplement component part such as *E. coli* 64G strain.

2.1. Research design

We conducted a prospective cohort experiment to study the features of the created semi-finished fish sausage according to its organoleptic

proprieties and effect *in vivo* on mice to confirm its safety and probiotic antagonistic action to the microbes of the *Enterobacteriaceae* family. All research stages are conducted according to the Veterinary Code of Conduct and the ethical guidelines for animal research established by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (reviewed by the Bioethics Committee at Kazakh National Agrarian Research University).

2.2. Study stages

2.2.1. Organoleptic features study

The consumer qualities of the developed product were assessed by organoleptic evaluation (GOST 9959-2015). Thus, the organoleptic analysis evaluated points such as the consumption view (the external view), the sectional view and the consistency with an assessment of the product. The commission formed the overall rating according to the profile analysis and scoring system.

The weighting coefficients were determined using the rank method and a survey of experts. This made it possible to determine the sequence of the significance of product quality indicators. The experts rated the quality indicators depending on their relevance. The quality indicators were combined with determination following principles as the arithmetic mean and a weighted average (Kuznetsova et al., 2022). An arithmetic weighted average was used to take into account the weighting coefficients of the quality indicators for semi-smoked sausages.

2.2.2. Sausage histological organization study

The histological examination was carried out to determine the product's microscopic structure and assess the state of its structural components against GOST R 19496-93 "Meat Method of histological examination" standard used in the experimentation.

10% neutral buffered formalin was used to fix histological specimens. After that, serial paraffin sections of 5–6 µm in thickness were cut with HESTION ERM-3100 semi-automatic microtome and MS-2 and subsequently stained with hematoxylin and eosin on Leica ST4040/#000000358 Stainer (BioLine LLC, St. Petersburg, the Russian Federation). Finally, the sausage structure was examined at 40× magnification using an MBI-6 microscope.

2.2.3. Animal studies

Two parallel experiments were conducted to investigate the effectiveness and safety of the proposed functional product.

For the first experiment (*the product effect study on living organisms*), four groups of 20 white outbred laboratory mice, each weighing 25–35 g, were formed. Three experimental groups were each fed a functional product containing different concentrations of the probiotic strain *E. coli* 64G: 5×10^9 CFU, 10^{10} CFU, and 2×10^{10} CFU, respectively. The fourth (control) group received a semi-finished product without a probiotic. The animals were observed for 21 days, with the analysis of their behavior and survival. Their hematological parameters (levels of white blood cells (WBCs), red blood cells (RBCs) and hemoglobin) were measured to assess their general condition.

The second experiment (*the preventive activity of the proposed functional product study*) included five random groups, each consisting of 20 1.8 months-old mice weighing 25–35 g. Four groups of mice were fed a sausage containing *E. coli* 64G strain, the probiotic properties of which were previously studied and tested. The fifth group received a sausage without a probiotic component (a 20 mL dose of saline solution was added instead). Further, each animal group was experimentally infected with virulent cultures of Enterobacteriaceae (*E. coli* 25 strain, *Salmonella typhimurium* 371 strain, and *Klebsiella pneumoniae* 30 strain).

Geriatric-diet-oriented semi-finished product from Nile tilapia fillet infected with *Escherichia coli* 64G strain was fed to each group in single doses of 3×10^9 CFU (group I), 5×10^9 CFU (group II), 10^{10} CFU (group III), and 2×10^{10} (group IV). Later (after 30 min), all five groups of animals were given orally 20 mL of a virulent culture. The animals were observed for 10 days.

A similar experiment was conducted under the demonstrated scheme for all pathogenic cultures (*E. coli* 25 strain, *S. typhimurium* 371 strain, and *K. pneumoniae* 30 strain).

Laboratory tests were performed as part of the experiment at the Department of Sanitary and Veterinary Expertise of Kazakh National Research Agrarian University and at Almaty Technological University, the laboratory of Kazakhstan-Japan Innovation Center and using the research base of AsylTasEngineering LLP.

At the end of the experiment, mice were sacrificed for macroscopic assessment internal organ function: heart, lungs, liver, spleen, kidneys, and stomach, and also subjected to microscopic examination.

2.3. Research results processing

There were three replicates for each of the experiments in our study to obtain a significant result. The accuracy of experimental results was assessed by calculating the statistical reliability of measurements ($p \leq 0.05$) using Microsoft Office Excel and Statistika 6.0 software packages.

3. Results

Based on the study's objectives, our task was to conduct a comparative assessment of the previously developed functional product against the control sample from the flesh fish of tilapia grown without using a probiotic component.

3.1. Histological features of the proposed product

Sausages made from the control group fillet had a uniform pink-red color, an elastic and juicy texture, a distinct pleasant taste, and a smell of aromatic spices. The functional product developed (Figure 1) from tilapia fillet cultivated using Enterocol had the following organoleptic qualities: wet and shiny surface, non-visualized individual fibers, and inclusions. The texture of the sausage was soft and juicy. The product had a pleasant, slightly salty taste with a distinct smell of spices. The section shows small pieces of meat in the product (Figure 1).

The average content of muscle tissue in sausage products from tilapia fillets was 40.2%, connective tissue—29.4%, and fat—14.7%. Carbohydrates and plant proteins accounted for 15.7%.

As the structure of muscle tissue in meat products is dependent on the production technology, comparative histology of sausages was conducted.

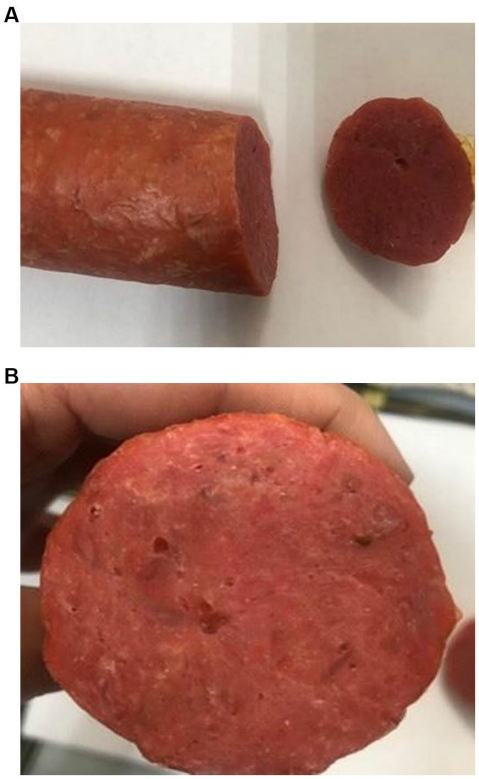

FIGURE 1
Visual assessment of the developed functional product: **(A)** section; **(B)** close-up section.

Muscle tissue was visualized in the experimental product's cross-section and longitudinal section. Muscle fibers were fragmented; nuclei and transverse striations were not found. Much of the muscle tissue was homogenized with visualization of vacuoles of various sizes. The connective tissue was prominently expressed. Fat was not visualized due to its extraction by organic solvents in the preparation of histological sections (Figure 2).

A significant portion of the fish sausage was homogenized in the control product with a single vacuole content of different sizes. Clusters of starch were observed. The fibers in the sample were fragmented. Vacuoles of different sizes and thin layers of connective tissue found in fat inclusions gave the section a reticulated appearance (Figure 3).

3.2. Sausage organoleptic study

Significant quality indicators for raw smoked sausages were evaluated in Table 1.

Thus, the most significant (significant quality indicator) in assessing raw smoked sausages are taste (0.27), aroma (0.24), texture and color, somewhat less significant (0.17), and the values of the weight coefficient of external species and sectional views are small.

When comparing the control and experimental sausages, it can be seen that the experimental sample has better consumer properties than the control sample (Table 2).

According to the table's two results, we can conclude that the average values of the proposed product exceed the values of the control sample in its organoleptic parameters such as appearance, color (nature, without artificial color adding), aroma, taste, and consistency.

In summary, the organoleptic and histological characteristics of the experimentally produced product allow us to say that the developed product has good consumer qualities. This provides us with a perspective to improve and develop our produced food. In addition, the *E. coli* 64G strain's effect on living organisms was studied to assess the probiotic (functional) qualities of the Enterocol drug as the stimulator in growing the fish base material. For this purpose, white mice were used as model organisms.

3.3. *In vivo* probiotic product's proprieties study

3.3.1. Safety of the proposed semi-finished food study

The analysis of the effect of the developed probiotic functional product on living organisms confirmed its safety. 100% of all animals (including both control and experimental groups) were active, with insignificant differences in the behavior among the groups and the absence of deaths. The analysis of hematological parameters (Figure 4) revealed that the immunological response to the functional product depends on the concentration of the probiotic component contained in it: Test 1—5×10^9 CFU; Test 2—10^{10} CFU; and Test 3—2×10^{10} CFU.

The analysis of the hematological parameters of mice during the experiment shows a stimulating effect of the developed product, which is reduced to the pre-experimental level when the probiotic supplement feeding is stopped. The 10^{10} CFU concentration of the *E. coli* 64G strain was found the have the most stimulating effect on a living organism.

3.3.2. Antagonistic action study of the Enterobacteriaceae family bacteria

The next stage of the experiment confirmed the preventive and antagonistic effect of the developed functional product on the opportunistic Enterobacteriaceae genera which cause intestinal infections.

Thus, the survival rate of animals infected with pathogenic Enterobacteriaceae in experimental groups was 85%–100% against 100% mortality observed in the control group (Table 3).

At the end of the observation of experimental and control groups, all animals were humanely euthanized and necropsied. The results of the pathoanatomical study revealed no pathological changes in the organs and tissues of experimental animals. All the examined organs and tissues were found within normal parameters (shape, size, volume, and texture).

4. Discussion

4.1. Study of sausage with probiotic component

The paper investigates a semi-finished fish product developed as a functional food in the form of a crude smoked fish sausage and the research conducted to study the product's attributes.

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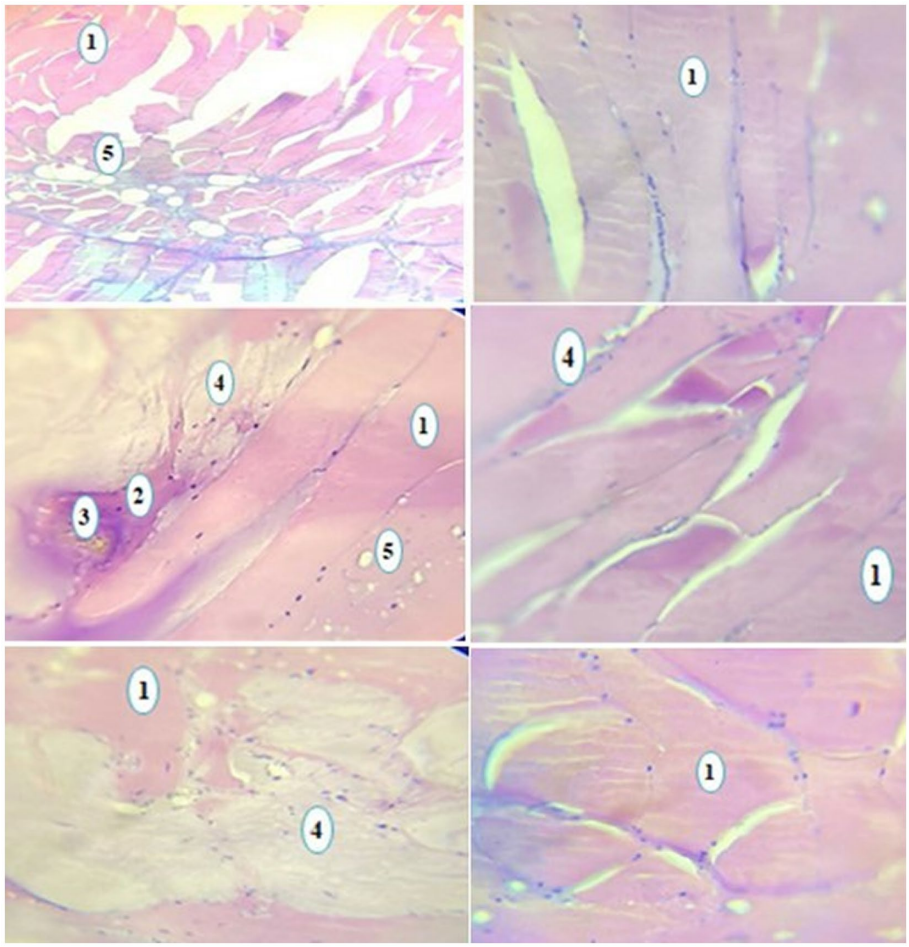

FIGURE 2
Cross-section and longitudinal section of sausage from tilapia cultivated using Enterocol. 40× magnification. Hematoxylin and eosin staining. 1—muscle fibers, 2—starch cluster, 3—adipose tissue, 4—connective tissue, 5—vacuole in minced meat.

4.1.1. The histological study of sausage structure

A probiotic-containing product was obtained by adding the probiotic component to the minced fish. It should be noted that the probiotic ingredient (Enterocol) has also been used experimentally as a feed additive for fish raised in closed reservoirs as part of commercial tilapia farming. (Zhakupova et al., 2017; Sarsembayeva et al., 2019; Sarsembaeva et al., 2021a). Our research results allow us to declare that using Enterocol as a food supplement component improves the quality of the fish flesh that clearly influence a sausage structure and, as a result, its consuming features. Comparing the sausages of the control (the fish base was obtained without Enterocol used in its feeding) and the experimental sausage recipes at the histology level proved these results (Figures 2, 3).

4.2. Sausages' organoleptic features

Global practice shows that numerous microorganisms, such as *Lactobacillus*, *Bifidobacterium*, and *Bacillus subtilis*, are used for manufacturing oral probiotics. However, they should have some properties, including acid and bile tolerance, safety, antibiotic resistance, non-toxicity, the ability to colonize host mucous membranes, and antagonistic activity (Zhou Y, et al., 2020). Khajavi et al. (2020) proposed *Bacillus* spp. for adding to meat food as the most stable bacteria in the technological processing phases. A composite probiotic component [*Streptococcus diacetilactis*, *Lactobacillus celobiosum* and *E. coli* (strain 64G)] was added to our product. We can conclude that this change has not damaged the proprieties of semi-finished fish sausage (Tables 1, 2).

Khajavi et al. (2020) also highlighted several challenges facing functional meat product developers, namely reducing or eliminating specific components such as fat and sodium. Ursachi et al. (2020) added nitrate/nitrite and N-nitrosamines to this list. They also noted that meat production quality could improve by using natural bioactive plant ingredients (antioxidants or dietary fibers) and probiotics. But in this case, there is another task—the recipe development that allows adding the probiotic component and remains the consumer proprieties of the developed product (Khajavi et al., 2020; Ursachi et al., 2020). In summary, our research has developed a successful recipe for functional fish semi-finished

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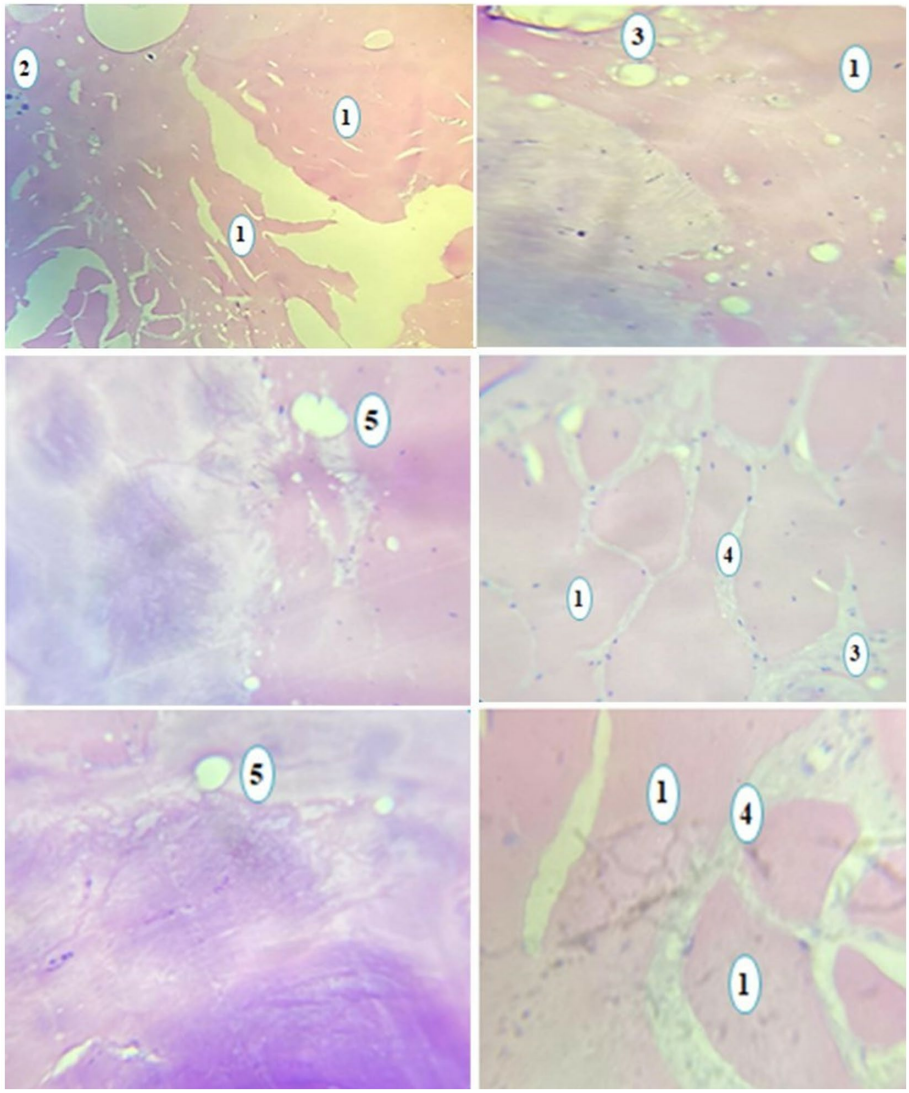

FIGURE 3
Cross-section of Enterocol-free tilapia sausage. 40× magnification. Hematoxylin and eosin staining. 1—muscle fibers, 2—starch cluster, 3—adipose tissue, 4—connective tissue, 5—vacuole in minced meat.

TABLE 1 Evaluation of the organoleptic characteristics of the proposed fish sausages.

Evaluated parameter	Tested sample number									Ranges' sum	Ponderability coefficient
	1	2	3	4	5	6	7	8	9		
Taste	5	4	4	4	4	5	4	4	5	39	0.30
Smell	4	5	5	4	4	5	4	4	5	40	0.28
Consistency	4	4	4	4	4		5	4	4	33	0.22
Color	4	5	4	5	5	5	4	4	5	35	0.22
Sectional view	4	4	4	4	5	5	4	5	4	39	0.11
External view	4	5	4	4	5	5	5	4	4	40	0.9
A sum of all ranges of quality characteristic										226	2.0

TABLE 2 The comparison assessments' summed results between the control and experimentally-created sausage variants.

Sausage variant	Appearance	Color in sectional	Smell	Taste	Consistency	Color	Average value
Control	0.48	0.73	1.97	2.16	1.41	1.36	1.37
Experimental	0.52	0.76	2.11	2.38	1.50	1.46	1.46

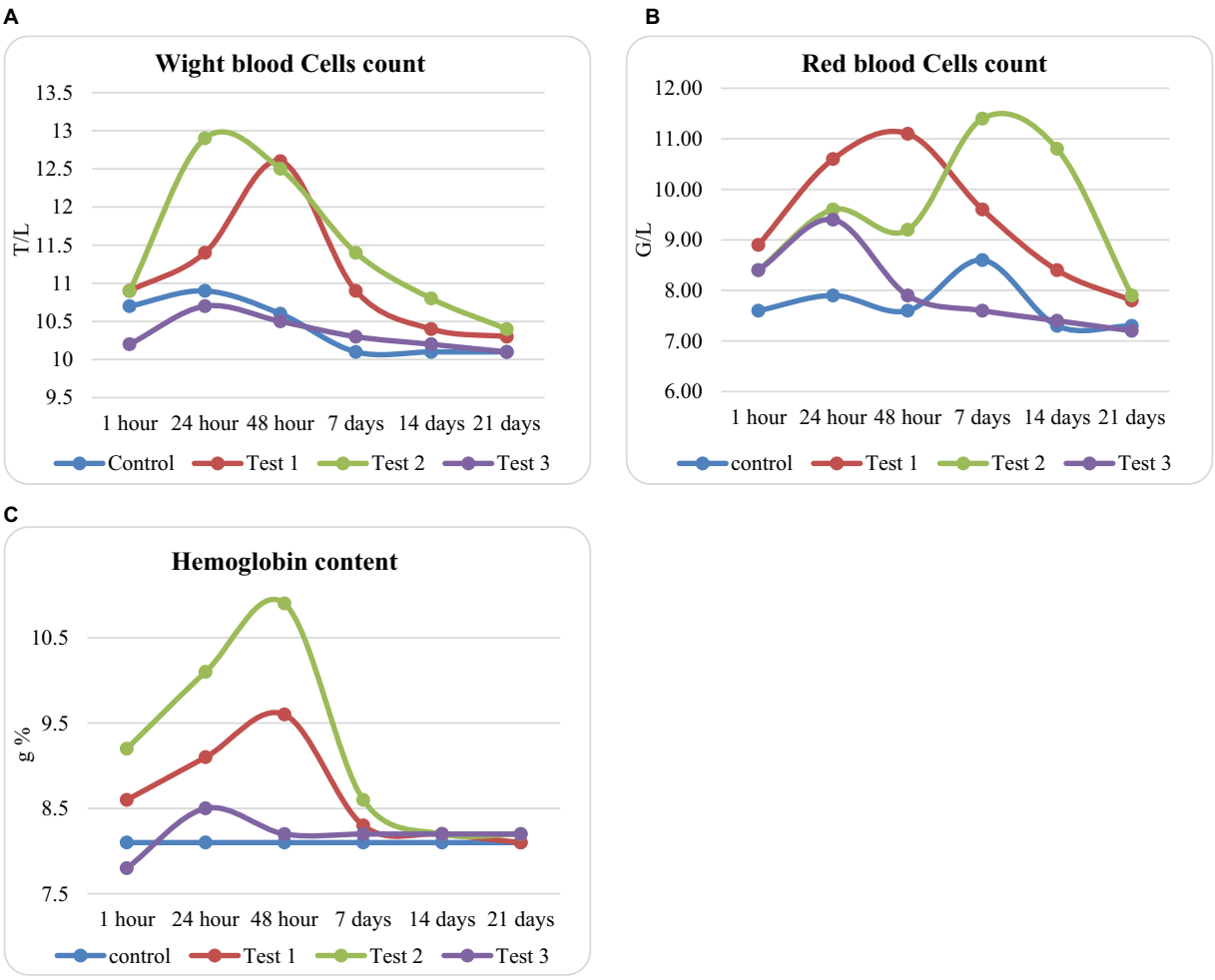

FIGURE 4
Hematological parameters of mice after receiving a single dose of the functional product: **(A)** white blood cells count; **(B)** red blood cells count; **(C)** hemoglobin content. The average content of muscle tissue in sausage products from tilapia fillets was 40.2%, connective tissue −29.4%, and fat −14.7%. Carbohydrates and plant proteins accounted for 15.7%.

products with probiotics, which allowed us to discover low-fat fish and meat products with added probiotic components.

4.3. *In vivo* probiotic product's proprieties study: its safety and activity

Notably, the *E. coli* 64G strain, when entering the gastrointestinal tract with a food product, can survive and compete with pathogenic and opportunistic microorganisms (in our experiment—*Enterobacteriaceae* family). According to the experimental study results (Table 3), introducing the Enterocol strain to animals at different concentrations, followed by their infection with virulent cultures, ensured a survival rate from 85 to 100%, with 100% mortality in the control group animals.

Thus, we can say about the efficient activity of *E. coli* 64G as an antagonist against pure pathogenic cultures of *E. coli* 25, *S. typhimurium* 371, and *K. pneumoniae* 30. In addition, the Enterocol strain concentration of 10^{10} CFU was found to be the optimal preventive dose in mice, ensuring 100% survival.

The non-pathogenic and non-toxic nature of the included *E. coli* strain was confirmed by behavioral observation and hematological studies following oral administration of the drug added to the food product. In particular, a peak increase in WBC counts in the blood of

TABLE 3 The results of experimental infection of mice with virulent Enterobacteriaceae cultures.

Experiment group	Probiotic's CFU per a dose	*E. coli* 25		*S. typhimurium* 371		*K. pneumoniae* 30	
		Mortality, %	Survived, animals	Mortality, %	Survived, animals	Mortality, %	Survived, animals
Group I	3×10^9	5	19	15	17	5	19
Group II	5×10^9	5	19	0	20	0	20
Group III	10^{10}	0	20	0	20	0	20
Group IV	2×10^{10}	0	20	0	20	0	20
Group VI	0 (20 mL saline)	100	0	100	0	100	0

CFU, colony-forming units.

mice 24 h after feeding is a natural response to the antigenic load in the gastrointestinal tract (Abbas et al., 2019).

4.4. The proposed semi-finished fish product effect on the live organism

It is interesting to note that the number of RBCs increased after the consumption of the experimental food, thus increasing the hemoglobin value. Similarly, the increase in the blood cell counts indicates the stimulating effect of the studied strain on the bone marrow (Zaporozhan et al., 2002). Therefore, this case calls for longer and more thorough studies on the number of morphological blood components to explore the possibility of using the developed product in complex anemia therapy, including anemia in older adults.

Also, it should be noted that the product from *Oreochromis niloticus* containing the *E. coli* 64G strain-based probiotic component can be used in geriatric nutrition. Hence, the discussed fish sausage can become a broad-spectrum preventive product containing the probiotic necessary for older adults (Banning, 2011; Alexandri et al., 2022), which will reduce the percentage of antibiotic use due to its antagonistic activity against pathogenic Enterobacteriaceae.

5. Conclusion

The proposed crude smoked tilapia sausage with a probiotic component is a functional semi-finished product. It can be considered for geriatric nutrition and has good organoleptic properties. The bacteria in the probiotic component remain stable and viable for a long-time during storage of the semi-finished product. The *E. coli* 64G strain possesses antagonistic activity among pathogenic Enterobacteriaceae. This allowed the animals in the experimental groups to survive after the experimental infection. The maximum protective effect of *E. coli* 64G was obtained at a concentration of 10^{10} CFU. A similar bacteria concentration also produced the most stimulating effect on animals, indicated by blood parameters. The general physiological condition and behaviour of the mice after consuming the product containing the *E. coli* 64G strain did not differ from that of the control group, proving the strain's safety and non-toxicity. However, the stimulating activity of the product needs to be studied more thoroughly and comprehensively in future research.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Author contributions

DM: methodology, funding acquisition, and writing—original draft. YU and NS: supervision, project administration, and writing—review and editing. AI and BJ: software, formal analysis, and writing—original draft. AN: resources. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan, grant number 236-16-GK.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abbas, A. K., Lichtman, A. H., and Pillai, S. (2019). *Basic immunology: functions and disorders of the immune system* (6). Philadelphia, PA: Elsevier.

Alexandri, M., Kachrimanidou, V., Papapostolou, H., Papadaki, A., and Kopsahelis, N. (2022). Sustainable food systems: the case of functional compounds towards the development of clean label food products. *Foods* 11:2796. doi: 10.3390/foods11182796

Angeles, J. G. C., Villanueva, J. C., Uy, L. Y. C., Mercado, S. M. Q., Tsuchiya, M. C. L., Lado, J. P., et al. (2021). Legumes as functional food for cardiovascular disease. *Appl. Sci.* 11:5475. doi: 10.3390/app11125475

Arboleya, S., Watkins, C., Stanton, C., and Ross, R. P. (2016). Gut bifidobacteria populations in human health and aging. *Front. Microbiol.* 7:1204. doi: 10.3389/fmicb.2016.01204

Ashirova, Z. B., Kuzhantaeva, Z. Z., Abdrassulova, Z. T., Shaimerdenova, G. Z., and Atanbaeva, G. K. (2021). Studying phytochemical features of three Asteraceae herbs growing wild in Kazakhstan. *Floresta Ambiente* 28:e20210060. doi: 10.1590/2179-8087-FLORAM-2021-0060

Banning, M. (2011). Ageing and *C. difficile* infection—the immune and gastrointestinal impact. *Gastrointest. Nurs.* 9, 42–47. doi: 10.12968/gasn.2011.9.4.42

Battino, M., Forbes-Hernández, T. Y., Gasparrini, M., Afrin, S., Cianciosi, D., Zhang, J., et al. (2019). Relevance of functional foods in the Mediterranean diet: the role of olive oil, berries and honey in the prevention of cancer and cardiovascular diseases. *Crit. Rev. Food Sci. Nutr.* 59, 893–920. doi: 10.1080/10408398.2018.1526165

Campmans-Kuijpers, M. J. E., and Dijkstra, G. (2021). Food and food groups in inflammatory bowel disease (IBD): the design of the Groningen anti-inflammatory diet (GrAID). *Nutrients* 13:1067. doi: 10.3390/nu13041067

Dyshluk, L. S., Dyshluk, L., Sukhikh, S., Sukhikh, S., Ivanova, S., Ivanova, S., et al. (2018). Prospects for using pine nut products in the dairy industry. *Foods Raw Mater.* 6, 264–280. doi: 10.21603/2308-4057-2018-2-264-280

Gok, I., and Ulu, E. K. (2019). Functional foods in Turkey: marketing, consumer awareness and regulatory aspects. *Nutr. Food Sci.* 49, 668–686. doi: 10.1108/NFS-07-2018-0198

Hutchinson, A. N., Tingö, L., and Brummer, R. J. (2020). The potential effects of probiotics and ω-3 fatty acids on chronic low-grade inflammation. *Nutrients* 12:2402. doi: 10.3390/nu12082402

Katsaga, A., Kulzhanov, M., Karanikolos, M., and Rechel, B. (2012). "Kazakhstan: health system review," in *Health systems in transition*, 14. Copenhagen, Denmark: European Observatory on Health Systems and Policies. Available at: https://apps.who.int/iris/bitstream/handle/10665/332396/HiT-14-4-2012-rus.pdf?sequence=1&isAllowed=y (Accessed March 10, 2023)

Khajavi, M. Z., Abhari, K., Barzegar, F., and Hosseini, H. (2020). Functional meat products: the new consumer's demand. *Curr. Nutr. Food Sci.* 16, 260–267. doi: 10.2174/1573401315666190227161051

Kuznetsova, T. G., Lazarev, A. A., Derevitzkaya, O. K., and Soldatova, N. E. (2022). The method of sensory evaluation of cooked sausage using a reference profile. *IOP Conf. Ser.: Earth Environ. Sci.*, 1052,:012123, doi: 10.1088/1755-1315/1052/1/012123

Lorimer, J. (2020). *The probiotic planet: using life to manage life*. Minneapolis, MN: The University of Minnesota Press.

Lyra, A., Hillilä, M., Huttunen, T., Männikkö, S., Taalikka, M., Tennilä, J., et al. (2016). Irritable bowel syndrome symptom severity improves equally with probiotic and placebo. *World J. Gastroenterol.* 22, 10631–10642. doi: 10.3748/wjg.v22.i48.10631

Sarsembaeva, N. B., Biltebai, A. N., Moldagalieva, D. Z., Urkimbaeva, A. E., and Kadirov, Z. N. (2021a). Sposob Povisheniya Produktivnosti Zhivotnikh [way to increase animal productivity]. KZ Patent No. 34283.

Sarsembaeva, N. B., Biyashev, B. K., Moldagalieva, D. Z., Biltebai, A. N., Makbuz, A. Z., Daribaeva, A. A., et al. (2021b). Sposob Proizvodstva Ribnikh Kolbas Gerodieticheskogo Naznacheniya [method for the production of fish sausages for geriatric purpose]. KZ Patent No. 34860.

Sarsembayeva, N. B., Moldagaliyeva, D. Z., Uzakov, Y. A. M., Abdigaliyeva, T. B., and Biltebay, A. N. (2019). Effects of new mixed feed based on nontraditional feed additives on fatty acid profile of tilapia (*Oreochromis niloticus*). *Int. J. Recent Technol. Eng.* 8, 3790–3794. doi: 10.35940/ijrte.A1362.078219

Talan, D. A., Moran, G. J., Newdow, M., Ong, S., Mower, W. R., Nakase, J. Y., et al. (2001). Etiology of bloody diarrhea among patients presenting to United States emergency departments: prevalence of *Escherichia coli* O157:H7 and other enteropathogens. *Clin. Infect. Dis.* 32, 573–580. doi: 10.1086/318718

Ursachi, C. Ș., Perța-Crișan, S., and Munteanu, F. D. (2020). Strategies to improve meat products' quality. *Foods* 9:1883. doi: 10.3390/foods9121883

Young, K. (2009). Omega-6 (n-6) and omega-3 (n-3) fatty acids in tilapia and human health: a review. *Int. J. Food Sci. Nutr.* 60, 203–211. doi: 10.1080/09637480903140503

Zaki, M. E. S., Alsayed, M. A. L., and Shrief, R. (2020). Study of the diagnostic value of interleukin-6 and interleukin-8 in children with acute gastroenteritis. *Germs* 10, 27–33. doi: 10.18683/germs.2020.1182

Zaporozhan, V. M., Napkhaniuk, V. K., Horianova, N. O., Bazhora, Y. I., Kresiun, V. Y., and Servetskyi, K. L. (2002). *Morfolohiia Klityn Krovi Laboratornykh Tvaryn i Liudyny: Atlas [morphology of blood cells of laboratory animals and humans: Atlas]*. Odesa, Ukraine: Odessa National Medical University.

Zhakupova, A. A., Biyashev, K. B., Biyashev, B. K., Ermagambetova, S. E., and Nurgozhayeva, G. M. (2017). Performance test of the drug "Enterocol". *J. Pharm. Sci. Res.* 9, 1941–1942. Available at: https://www.kaznaru.edu.kz/file_archive/3502539439var_993.pdf (Accessed March 10, 2023)

Zhou, C., Fang, X., Xu, J., Gao, J., Zhang, L., Zhao, J., et al. (2020). *Bifidobacterium longum* alleviates irritable bowel syndrome-related visceral hypersensitivity and microbiota dysbiosis via Paneth cell regulation. *Gut Microbes* 12:e1782156. doi: 10.1080/19490976.2020.1782156

Zhou, Y., Zeng, Z., Xu, Y., Ying, J., Wang, B., Majeed, M., et al. (2020). Application of *Bacillus coagulans* in animal husbandry and its underlying mechanisms. *Animals* 10:454. doi: 10.3390/ani10030454